CORRELATION BETWEEN SEVERITY OF ULCERATIVE COLITIS & CO-INFECTION WITH PARASITES, CYTOMEGALOVIRUS AND/OR *CLOSTRIDIUM DIFFICILE*

A dissertation submitted in part fulfillment of the requirements for DM (Branch IV, Gastroenterology) examination of the Tamil Nadu Dr.M.G.R. Medical University, Chennai to be held in August 2011

CERTIFICATE

This is to certify that this dissertation entitled **"Correlation between severity of ulcerative colitis and co-infection with parasites, cytomegalovirus and/or *Clostridium difficile*"** is a bonafide work done by Dr.Venkatakrishnan H. Iyer in partial fulfillment of rules and regulations for DM (Branch IV,Gastroenterology) examinations of the Tamil Nadu Dr. M.G.R. Medical University, Chennai to be held in August 2011

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INDEX

Topic **Page No**

INTRODUCTION

Ulcerative colitis (colitis ulcerosa, UC) is a chronic idiopathic inflammatory disease of the gastrointestinal tract that affects the large bowel and is a major disorder under the broad group of conditions termed *inflammatory bowel diseases* (IBDs).

Etiopathogenesis of ulcerative colitis largely remains hypothetical. It arises from a complex interaction between genetic, immune and environmental *factors*. Inflammation first affects the rectum and extends proximally along a variable length of the colon. In most of the cases, the disease follows a chronic relapsing, remitting course. Treatment is mainly immunosuppressive. Control of disease includes long term medical management and regular monitoring for complications.

Enteric infections are known to be associated with exacerbations of UC. India, being a tropical country has a high prevalence of enteric pathogens. There is scant literature from India on what are the interactions of enteric pathogens with disease severity of UC.

AIM OF THE STUDY

To perform a prospective cross-sectional observational study in patients with ulcerative colitis

- To determine whether there is any association between intestinal infection (with parasites, cytomegalovirus or *Clostridium difficile*) and clinical disease severity in patients with ulcerative colitis

REVIEW OF LITERATURE

HISTORY

Samuel Wilks, in 1859, is credited to be the first person to describe a condition which he called "Idiopathic colitis", that was distinct from the then more common bacillary dysentery[1]. He reported the pathologic finding of dilated and thinned colon with severe pancolonic inflammation in a patient with this condition[2]. In 1909, Hawkins described the chronic and relapsing nature of the disease course and the "stealthy hemorrhage" onset of distal disease, in which bleeding per rectum often occurred in the presence of constipation[3]. In that same year, Sir Arthur Hurst gave a more elaborate description of UC, including its sigmoidoscopic appearances and differentiation from bacillary dysentery[4]. In 1913, Brown proposed ileostomy as a treatment modality, diverting the fecal stream to allow ulcers to heal[5]. It was only in 1940 that sulfasalazine was introduced as a treatment modality for UC[6,7]. In 1950, Truelove and Witts proved steroids as effective medical treatment[8]. After the discovery of deoxyribonucleic acid (DNA) in 1953 by Watson and Crick[9], there was better understanding regarding the genetic and molecular basis of UC.

EPIDEMIOLOGY

The incidence and prevalence of UC vary with geographic location and ethnicity. Rigorous epidemiologic studies have been limited by several potential issues: Diagnosis of UC may be difficult due to its varied clinical manifestations and, in some regions, the common occurrence of infectious colitis that can mimic UC. Prevalence and incidence in India have been estimated as 44.3 per 100000 and 6.0 per 100000 population per year respectively[10]

UC can occur at any age, although diagnosis before the age of five years or after 75 years is uncommon. The peak incidence of UC occurs in the second and third decades of life. Studies

have reported a second, smaller peak in the elderly, between the ages of 60 and 70 years. This second peak of disease incidence is less pronounced than that for Crohn's disease. Most studies have not shown any gender difference in the occurrence of UC, and a male-to-female ratio of nearly 1:1 applies to all age groups. The incidence and prevalence also varies according to ethnicity. The highest incidence is seen in the Jewish population.

ETIOLOGY AND PATHOGENESIS

The current understanding regarding the pathogenesis involves a complex interaction of three elements: genetic susceptibility, host immunity, and environmental factors. Loss of immune tolerance in a genetically predisposed individual leads to development of acute and chronic inflammation which causes mucosal damage. The candidates for specific inciting antigens causing the inflammatory response include pathogenic and commensal microorganisms, metabolic byproducts of these agents and their normal epithelial structures.

GENETICS

Family History

Genetic factors predispose to UC, this is supported by familial incidence of UC of about 10% to 20% of patients, who have at least one other affected family member[11]. As per evidence from European twin studies, around 6-16% of monozygotic twin pairs had concordant UC in comparison to 0-5% of dizygotic twin pairs[12-14].

Familial association is greater in persons of Jewish descent, a heritage known to have a higher incidence of IBD. The lifetime risk of developing disease is three-fold higher among first-degree relatives of Jewish patients compared with relatives of non-Jewish patients[15].

Genetic Mutations

The inheritance of UC is complex and polygenic. Linkage studies have suggested that there are susceptibility genes for UC on chromosomes 1, 2, 3, 5, 6, 7, 10, 12, and 17[16-19].The IBD2 locus on chromosome 12 appears to have strong linkage demonstrated in studies involving large numbers of families with UC[18]. The C3435T polymorphism for the human multidrug resistance 1 *(MDR1)* gene is linked to susceptibility for UC but not Crohn's disease[20].

A genome-wide association study showed strong association between the gene encoding IL-23R and both Crohn's disease and UC[21]. IL-23R plays a key role in the differentiation of a relatively newly discovered subset of T cells called Th17 cells.

There also are genes that seem to influence disease. The best studied of these genes are the human leukocyte antigen (HLA) alleles. HLA-DR2 (*DRB1*1502*) appears to be involved in disease susceptibility in Japanese and Jewish populations. An association between severe disease and a rare allele of HLA-DR1 (*DRB1*0103*) has been reported. In some studies, the HLA-DR3,DQ2 haplotype is associated with extensive colitis, particularly in women.

ENVIRONMENTAL FACTORS

It is now almost universally accepted that the pathogenesis of IBD is a result of continuous antigenic stimulation by commensal enteric bacteria, fungi, or viruses, leading to chronic inflammation in genetically predisposed hosts having defects in mucosal barrier function, microbial killing, or immunoregulation. No specific infective organism has been incriminated till date.

The fact that both UC and Crohn’s disease preferentially occur in regions of the bowel that contain the highest concentration of bacteria, namely, the terminal ileum and the colon, where

bacterial concentrations approach 10^{12} organisms per gram of luminal contents, suggests that intestinal organisms could play a role. A diversion of fecal stream in patients with inflammatory bowel disease can treat and even prevent disease, whereas reinfusion of ileostomy contents leads to new inflammatory changes within only one week[22]. Data show that antibiotics are useful in the treatment or postsurgical prevention of Crohn's disease and pouchitis. Probiotics have been demonstrated to be effective in the primary and secondary prevention of pouchitis. The gnotobiotic (germ-free) rodent model reveal that without intestinal microflora, the rats remain healthy, but with intestinal colonization, they develop intestinal inflammation.[35-38] Antibiotics and probiotics have been demonstrated to be useful in this model.[39,40]

Recently, a lot of knowledge has been gained about the human gastrointestinal microbiome. There are at least 1800 genera and between 15,000 and 36,000 species of bacteria[23-25]; more than 45,000 bacterial small-subunit (SSU) rRNA genes have been identified[23-25]. Of the bacterial genes recognized till date, almost all (more than 98%) can be classified into four phyla[25]:

- Firmicutes (64%); includes the family Lachnospiraceae (e.g., *Clostridium* groups XIVa and IV) and the subgroup *Bacillus* (e.g., Streptococcaceae and Lactobacillales)
- Bacteroidetes (23%)
- Proteobacteria (8%); include the family Enterobacteriaceae (e.g., *Escherichia coli*)
- Actinobacteria(3%)

Four mechanisms have been hypothesized to explain how the normal intestinal microbiome might initiate or contribute to the development of the chronic inflammatory state[26]. First, microbes can trigger intestinal inflammation, either by adhering or invading intestinal epithelial cells, thereby initiating a cascade of proinflammatory cytokine production.

Second, an alteration in the balance between protective and harmful intestinal bacteria, termed *dysbiosis,* can lead to disease. Comparisons of intestinal microbiome in IBD with that in healthy controls show reduction in biodiversity in the IBD populations by 30% to 50%. One study found that this reduction in biodiversity was due to decreased concentrations of Firmicutes (specifically *Lachnospiraceae*) by 300-fold and *Bacteroides* by 50-fold[25]. The loss of these organisms is important because they are known to produce short-chain fatty acids, such as butyrate, which nourish colonocytes. As a result of their decrease, the relative concentrations of *Proteobacteria* and *Actinobacteria* increase in IBD patients relative to controls, although quantitative PCR analysis showed that the absolute numbers of *Enterobacteriaceae* were not higher in IBD patients than in controls. Loss of protective bacteria, however, could set the stage for overgrowth of pathogenic bacteria.

Third, genetic defects in host microbial killing or impaired mucosal barrier function could lead to immune hyper-responsiveness to intestinal bacteria, as the microbes have more exposure to epithelial cells and can trigger the production of high levels of proinflammatory cytokines. Fourth, genetic defects in host immunoregulation can lead to an abnormally high immune response to even nonpathogenic bacteria, by mechanisms of abnormal antigen processing or presentation, loss of tolerance, or overly aggressive T-cell responses.

In addition to infectious agents, several other environmental factors have been proposed as contributing etiologic factors of UC. Smoking and previous appendectomy are believed to be protective in UC.

IMMUNE FACTORS

Enteric immune response is incriminated to be an important factor in the pathogenesis of UC. Both humoral and cell-mediated responses are described to be abnormal.

Humoral Immunity

Tissue plasmacytosis is noted in the colon. Increased levels of IgG, predominantly IgG_1 and IgG_3 subclasses is noted[27,28]. Cross-reaction between microbial antigens and colonic epithelial epitopes may be an important event in pathogenesis. There is an increased association with other autoimmune disorders, namely thyroid disease, diabetes mellitus, and pernicious anemia.[53] The most well described intestinal auto-antigen is a 40-kDa epithelial antigen found in normal colonic epithelium[29]. The antibody response to this 40-kDa protein appears to be unique to UC. This autoantigen shares an epitope with antigens found in the skin, bile duct, eyes, and joints. This may explain the extraintestinal manifestations of UC. The exact significance of this autoantibody in UC, however, remains unclear presently. pANCA is another autoantibody, present in 60% to 85% of patients with UC[30,31]. It is synthesized within the lamina propria and is of the IgG_1 subclass. The current status of pANCA is that it has no pathogenic role in UC but that it might serve as a potential marker of susceptibility and genetically distinct subsets of UC. pANCA may be associated with a more-aggressive disease course[32] and with the development of pouchitis after ileal pouch-anal anastomosis (IPAA) in patients with UC[33,34]. pANCA seroreactivity is associated with a predominant colonic involvement in Crohn's disease[35].

Some antibodies to bacterial antigens have been identified in UC - anti-CBir1 and anti-OmpC. Anti-Cbir1 is an antibody to flagellin from *Clostridium* species; found in about 6% of UC patients[36] and appears to be related with the development of pouchitis[37]. Anti-CBir1 also is found in 50% of patients with Crohn's disease, in which it is associated with more complicated

disease[36]. Anti-OmpC (outer membrane porin C of *E. coli*) is seen more often in UC patients who have a mixed family history of Crohn's disease and UC rather than those with a family history of only UC[38].

Cellular Immunity

Immune dysregulation in UC affects both innate and adaptive components of cell-mediated immunity. The innate immune system provides the first line of non-specific defence against all foreign antigens. Pattern-recognition receptors (PRRs) are present on cell membranes, which comprise of the 11 Toll-like receptors (TLRs) and 23 nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) identified till date.

Antigen binding at the TLRs and NLRs results in a cascade of events, leading to a final common pathway of nuclear factor-κB (NF-κB) activation, which enhances the transcription of genes encoding for various proinflammatory cytokines (including TNF, IL-1, IL-6, and IL-8), chemokines, adhesion molecules, and costimulatory molecules. Activation of NF-κB also hastens the maturation of dendritic cells, that are involved in antigen presentation. Defects in any part of the innate immunity leads to abnormal bacterial processing and possibly IBD[39].

The adaptive immune system, comprising T cells and B cells, is specific. Lymphocytes are classified as per their locations in the mucosa: lamina propria lymphocytes and intraepithelial lymphocytes (IELs). Lamina propria lymphocytes express, α4β7, a surface adhesion molecule that provides a homing signal for peripheral immune cells to the mucosal sites[40]. Mucosal T cells within the lamina propria and epithelium, as well as peripheral blood T cells, display activation markers, suggesting an activated memory phenotype[41]. There is evidence that the T-cell receptor repertoire is altered in active IBD[42].

Epithelial Cells

Intestinal epithelial cells not only serve as a barrier, but also play a role in enteric immunity. Colonocytes express class II major histocompatibility complex (MHC) antigens and function as antigen-presenting cells[43]. They are functionally active and express cytokine receptors, secrete various cytokines and chemokines, and express leukocyte adhesion molecules[44-47].

Patients with UC have an increased turnover rate of colonic epithelium[48] and abnormalities of epithelial cells like reduced metabolism of short-chain fatty acids, especially butyrate, abnormal membrane permeability[49], and altered composition of glycoprotein mucus produced by the colonic epithelium[50]. The mucus layer in UC is thinner than normal[51]. These abnormalities can lead to increased numbers of adherent bacteria, in both the mucus layer and even at the epithelial surface, in patients with UC[52-54]. The role of epithelial cells in the pathogenesis of IBD is vindicated further by animal models of colitis produced by disruption of colonic epithelium[55].

Consequences of Immune Activation

$CD4^+$ T cells are classified into three major immune phenotypes: T helper 1 (Th1), T helper 2 (Th2), and a newly recognized subset called T helper 17 (Th17).

The Th1 response, initiated by IL-12 due to exposure to infectious agents is characterized by cell-mediated immunity and is associated with the production of interleukin (IL)-2 and interferon (IFN)-γ. The Th2 response is characterized by the production of cytokines IL-4, IL-5, IL-10, and IL-13, which amplify the humoral immune response. Th1 and Th2 subsets reciprocally down-regulate each other through cytokine production[56]. Th1 and Th2 pathways can be regulated by unique regulatory T cell (Treg) subsets that produce IL-10 and transforming growth factor (TGF)-β and down-regulate inflammation[57].

An oversimplification of adaptive immunity in IBD would be to say that Crohn's disease is mediated by Th1 cells whereas UC is mediated by Th2 cells; the true story is much more complex. Macrophages in the inflamed colon in UC produce IL-1β, TNF, and IL-6, whereas lamina propria T cells probably produce IL-2 and IFN-γ. Presentation of antigen to $CD4^+$ lymphocytes by colonic epithelial cells that express HLA class II antigens further enhances this immune response[43]. A specialized type of T cell, the natural killer (NK) T cell, seems to mediate the Th2 response in UC[58,59]. These NK T cells, unlike classical NK T cells do not express the typical NK T-cell receptors and secrete large amounts of IL-5 and IL-13, which are cytotoxic for intestinal epithelial cells.

A novel T-cell–mediated inflammatory pathway, the Th 17 pathway has been recently discovered. Th17 cells produce a variety of cytokines, mainly IL-6 and IL-17. IL-17 is a potent proinflammatory cytokine that facilitates T-cell activation and stimulates an array of cells, including fibroblasts, macrophages, epithelial cells, and endothelial cells, to produce a variety of proinflammatory cytokines like IL-1, IL-6, TNF-α, and chemokines[60]. Th17 lineage is inhibited by Th1 and Th2 cells but is promoted by IL-6, TGF-β, IL-21, and IL-23R[61]. IL-23R, which is highly expressed by activated Th17 cells, also is expressed by NK cells, NK T cells, other $CD4^+$ T cells, and $CD8^+$ T cells[19]. The interaction of IL-23 with its receptor has been shown to have a pivotal role in the development of inflammation in various mouse models of colitis[62,63]. Antibodies to IL-23 could be a potential therapeutic target in the future.

PSYCHOGENIC FACTORS

There is evidence that psychosocial stress increases the risk of relapse in patients with quiescent UC[64,65].

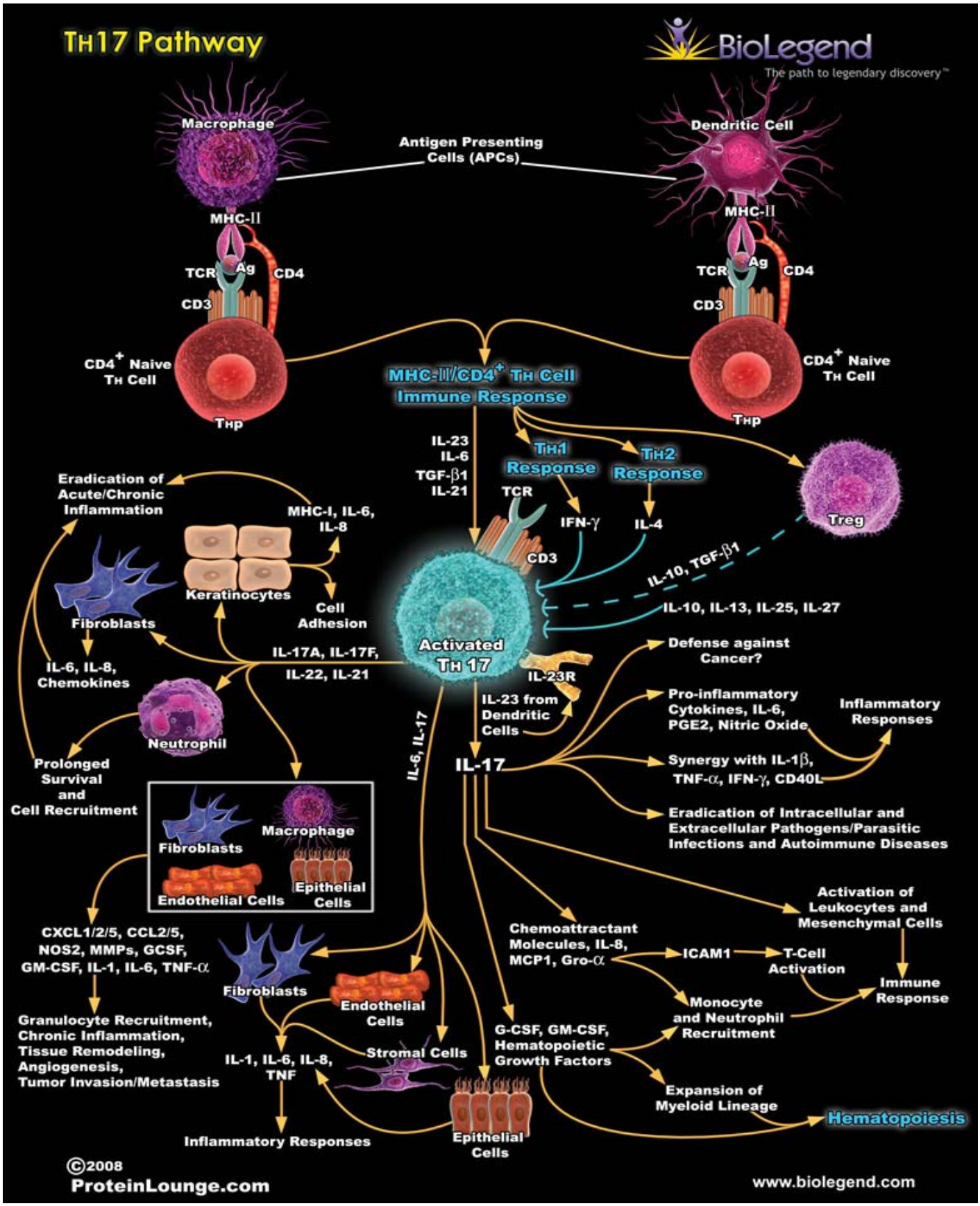


Fig 1: Immune pathways in ulcerative colitis.(Courtesy: www.biolegend.com)

PATHOLOGY

At initial presentation, approximately 45% have disease limited to the rectosigmoid, 35% have disease extending beyond the sigmoid but not involving the entire colon, and 20% of patients have pancolitis[66]. Typically, the disease is most severe distally and progressively less severe more proximally. Continuous and symmetrical involvement is the hallmark of UC. There is a sharp transition between diseased and uninvolved segments of the colon.

In two conditions, skip lesions can occur. First, when the patient is treated with topical enema leading to mucosal healing in the rectum and distal colon. Second, 75% of the patients with left sided UC have peri-appendiceal inflammation and patchy inflammation in the caecum.

Macroscopically, the mucosa appears hyperemic, edematous and granular in early disease, which progresses to visible punctuate ulcers with hemorrhagic mucosa. Ulcers enlarge and extend upto the lamina propria. Epithelial regeneration with recurrent attacks leads to a pseudopolyp like appearance. With long standing disease the colonic mucosa becomes atrophic and featureless.

Microscopically, the early stage of UC shows edema of the lamina propria with congestion of capillaries and venules, often with extravasation of red blood cells. Neutrophilic infiltration of colonic crypts causes cryptitis and ultimately to crypt abscesses with neutrophilic accumulations in crypt lumens. Inflammation is typically confined to mucosa and usually do not extend beyond the luminal aspect of muscularis mucosa. The classic histologic description of chronic quiescent UC is crypt architectural distortion or actual dropout of glands. Architectural changes include branching or bifid glands, wide separation among glands, and shortened glands that do not

extend down to the muscularis mucosa. Paneth cell metaplasia (Paneth cells located distal to the hepatic flexure, where they normally are absent) is another feature typical of UC.

CLINICAL FEATURES

Symptoms can range from diarrhea, rectal bleeding, passage of mucus, tenesmus, urgency, and abdominal pain. In severe cases, fever and weight loss may be prominent. The symptomatology differs according to the extent of disease[67]. Patients with proctitis predominantly present with tenesmus, urgency, mucus, and bleeding, whereas patients with extensive colitis present with more diarrhea, weight loss, fever, clinically significant blood loss, and abdominal pain. Up to 30% of patients with proctitis or proctosigmoiditis complain of constipation and hard stools[68]. Fever and tachycardia are associated with severe disease. Abdomen is usually soft with mild tenderness over the diseased segment.

DIAGNOSIS

Anemia, hypoproteinemia and elevation of inflammatory markers like ESR and CRP may be present with severe disease. A stool examination for parasites, faeces culture and toxin for *Clostridium difficile is important* to rule out infectious mimics of UC. Diagnosis is confirmed by colonoscopy and biopsy.

ASSESSMENT OF SEVERITY

Two assessment scores are popularly used in UC - Truelove and Witts classification and the Ulcerative Colitis Disease Activity Index (UCDAI). There are in addition, endoscopic and histologic scales in grading colitis.

Truelove and Witts Classification of the Severity of Ulcerative Colitis[69]

Mild
<4 stools/day, without or with only small amounts of blood No fever No tachycardia Mild anemia ESR < 30 mm/hr
Moderate
Intermediate between mild and severe
Severe
>6 stools/day, with blood Fever > 37.5?C Heart rate > 90 beats/min Anemia with hemoglobin level < 75% of normal ESR > 30 mm/hr

Endoscopic and Histologic Assessment of Disease Activity in Ulcerative Colitis[70,71]

SCORE	**CRITERIA**
Endoscopic Assessment	
0	Normal mucosa
1	Loss of vascular pattern
2	Granular, nonfriable mucosa
3	Friability on rubbing
4	Spontaneous bleeding, ulceration
Histologic Assessment	
0	Normal
1	No significant inflammation: Possibly architectural changes of chronic disease and small foci of lymphocytes but no acute inflammation, crypt abscesses, or epithelial destruction
2	Mild to moderate inflammation: Edema, vascularity, increased acute and chronic inflammatory cells but intact epithelium
3	Severe inflammation: Heavy infiltrate of acute and chronic inflammatory cells, crypt abscesses, ulceration of surface epithelium, purulent exudate

Ulcerative Colitis Disease Activity Index[72]

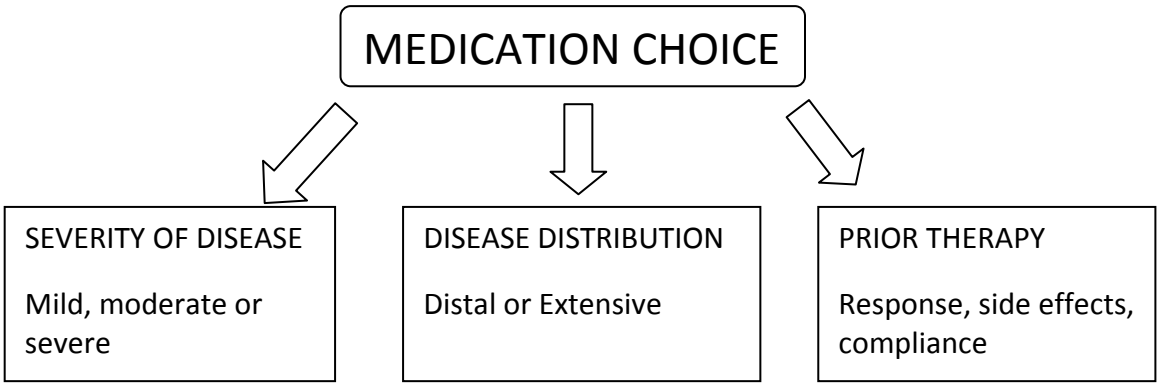
SCORE	CRITERIA
Stool Frequency	
0	Normal
1	1-2 stools/day > normal
2	3-4 stools/day > normal
3	>4 stools/day > normal
Rectal Bleeding	
0	None
1	Streaks of blood
2	Obvious blood
3	Mostly blood
Mucosal Appearance	
0	Normal
1	Mild friability
2	Moderate friability
3	Exudation, spontaneous bleeding
Physician Global Assessment	
0	Normal
1	Mild
2	Moderate
3	Severe

* Sutherland index: Range, 0-12

None of these disease activity instruments has ever been formally validated. Generally, a patient is considered to be in remission if the UCDAI score is 2 or less and to have severe disease if the score is greater than 10. A decrease in three points from the patient's initial baseline score reflects a clinical response. An index very similar to the UCDAI that has been used extensively in recent randomized, controlled trials (RCTs) is the Mayo score, which incorporates the same four components as the UCDAI

TREATMENT

Treatment depends on various factors



Induction Therapy for Ulcerative Colitis Based on Disease Severity

Mild Disease
5-Aminosalicylates Topical (distal colitis) Oral (distal/extensive colitis) Combination
Moderate Disease
5-Aminosalicylates Topical (distal colitis) Oral (distal/extensive colitis) Combination Glucocorticoids Topical (distal colitis) Oral (distal/extensive colitis) Combination Azathioprine or 6-mercaptopurine
Severe Disease
IV glucocorticoids IV cyclosporine IV infliximab

EXTRAINTESTINAL MANIFESTATIONS

Common Extraintestinal Manifestations of Ulcerative Colitis

Cutaneous/Oral
Angular stomatitis
Aphthous stomatitis
Erythema nodosum
Oral ulcerations
Psoriasis
Pyoderma gangrenosum
Pyostomatitis vegetans
Sweet's syndrome (acute febrile neutrophilic dermatosis)
Ophthalmologic
Conjunctivitis
Episcleritis
Retinal vascular disease
Scleritis
Uveitis, iritis
Musculoskeletal
Ankylosing spondylitis
Osteomalacia
Osteonecrosis
Osteopenia
Osteoporosis
Peripheral arthropathy
Sacroiliitis

Hepatobiliary
Autoimmune hepatitis
Cholangiocarcinoma
Pericholangitis
Primary sclerosing cholangitis
Hepatic steatosis
Hematologic
Anemia of chronic disease
Autoimmune hemolytic anemia
Hypercoagulable state
Iron deficiency anemia
Leukocytosis or thrombocytosis
Leukopenia or thrombocytopenia

INTERACTIONS OF PARASITES, CMV AND CLOSTRIDIUM DIFFICILE WITH UC

Evidence in the literature do not point to any specific infection as a causative mechanism in IBD, but indicate that gut pathogens could cause trigger the onset of IBD and are associated with aggravation of quiescent disease. Though these infections are self limited, they initiate an inflammatory cascade, which lead to a series of events leading to chronic disease in a genetically susceptible host ('hit-and-run' hypothesis). Epidemiological and microbiologic studies suggest that enteropathogenic microorganisms play an important role in the clinical initiation and relapses of IBD. Thus, for optimal medical treatment, microbiologic screening is helpful in patients with flares of IBD.

A recent report from AIIMS found that 12% of patients with active UC had parasites, 8% had CMV and 10% had HSV infection[73]. The clinical presentation of an acute episode in a majority of UC patients is indistinguishable from enteric infection. Patients with ulcerative colitis often have intermittent flares alternating with periods of quiescent disease. Intestinal infections may be responsible for flares in some of these patients. Of the intestinal parasites, infection with *Entamoeba histolytica* and *Strongyloides stercoralis* is likely to cause flares in ulcerative colitis. Less commonly recognized are infection with cytomegalovirus and *Clostridium difficile*. In a study from Chandigarh, *C. difficile* toxin was detected in the stool of 13% of patients with UC[74]. In an earlier study from the same centre, 32% of active UC patients had parasitic or bacterial pathogens, compared to 4% of inactive UC patients[75].

Treatment of ulcerative colitis flare is essentially immunosuppressive. It is very important to recognize an underlying enteropathogen, prior to hiking the immunosuppression regime of the patient. Prompt diagnosis and treatment of infection can prevent unnecessary use of steroids and

immunosuppressive therapy. Enteric infections are common in tropical countries like India. Thus, there is still the chance that enteric pathogens could alter the natural history of UC.

A large population based cohort study showed that the use of biologic agents does not seem to increase the risk for *Clostridium difficile* infection(CDI)[76]. Recently published single-center studies and US inpatient database studies reported increasing rates of CDI among IBD patients and their role in an increased rate of hospitalizations and mortality[77-80]. The risk of CDI in IBD patients appears to persist even after colectomy. CDI can involve the small bowel[81]. CDI has also been reported in UC patients with restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA)[82,83].

Short and long-term outcomes with *Clostridium difficile* infection and inflammatory bowel disease[84]

Short-term outcomes

Toxic megacolon

Colonic perforation

Peritonitis with sepsis

? Increased hospitalization duration and costs

Colectomy rates highly variable

Long-term outcomes

Increased UC related hospitalization and emergency room visits

? Escalation of medical treatment

Increased rate of colectomy

Table 1: Differentiating Clostridium difficile infection and inflammatory bowel disease[84]

Features	**Isolated CDI**	**CDI and IBD**
Setting	Often hospital acquired	Often community acquired
Risk factors	• Antibiotic exposure prior to infection common • Immunomodulator & corticosteroid use • Increasing age	• Many patients lack history of antibiotic exposure • Immunomodulator and corticosteroid use playing even a greater role • Increasing age • Risk greater with UC than with CD, greater with colonic involvement than with small bowel
Clinical features	Watery diarrhea	Bloody or mucoid diarrhea
Short term outcome	Complications include toxic megacolon, perforation, peritonitis with sepsis	Similar to patients without IBD Hospital costs and length of stay variable in studies Increased mortality in some studies Risk of colectomy unclear
Long term outcome	-	Unclear; increase in hospitalization, increased medication usage and colectomy rates noted in retrospective studies
Diagnosis	ELISA testing for stool testing	ELISA may be less sensitive
Endoscopy	Pseudomembranes seen	Pseudomembranes rare
Treatment	Metronidazole for mild to moderate disease Vancomycin for severe disease	?Vancomycin for any hospitalized patient
Recurrence	20% after 1st episode	Highly variable 10-58%, may be higher
Extra-colonic involvement	Small bowel can be affected	Most cases of IBD have small bowel involvement; pouchitis can also occur

Although a variety of tests are available for the diagnosis of *Clostridium difficile* infection, ELISA for stool toxin is the most commonly performed test. ELISA is relatively inexpensive, easily available and technically less cumbersome than other techniques. It is based on the detection of toxin A and/or B in the stool using either a monoclonal antibody or a polyclonal antiserum that recognizes the specific toxin. Results are available within 2-6 hours. Most ELISAs for detection of both toxins A and B in stool have a sensitivity of 70-90% compared to culture of *Clostridium difficile*. Upto 30% of tests may be falsely negative, considering tissue cytotoxicity assay or culture as gold standard[85,86]. Specificity is very high (99%)[86,87]. Lower sensitivity of ELISA test can be improved by performing it on 2 or 3 consecutive specimens rather than on 1 specimen, which increases the diagnostic yield by 5%-10%[88]. The diagnostic yield of ELISA testing may be much lower in IBD patients. Four sequential stool samples were shown to increase the diagnostic yield to 92%[89].

Cell cytotoxicity assay is the current gold standard test for diagnosis of CDI. It detects as low as 10 picograms of toxin and is the most sensitive test for detection of toxin B[90-93]. It is based on the principle that the toxins in the stool exert a cytopathic effect characterized by cell rounding which can be demonstrated in tissue culture. The test is highly sensitive (94%-100%) and specific (99%). Disadvantages are its relatively high technical expertise and the 24-48 h needed to complete the assay[94]. Stool culture is seldom used for routine diagnosis because of labor intensiveness, long turnaround time (24-48 h) and a low specificity. culture permits molecular typing of the organisms, it is essential for monitoring molecular epidemiology and antibiotic susceptibility[90].

Polymerase chain reaction (PCR) based primers for the detection of genes for toxins A are available and this test is highly sensitive and specific for the diagnosis of CDI[95,96]. Culture of the

organisms may be required for PCR, which makes the process more technically demanding and challenging. A study based on the nested PCR assay reported a 99% concordance with the cytotoxicity assay and a sensitivity of 96.3% and a specificity of 100%[96].

In patients with IBD who present with worsening symptoms, CDI should be thought of and ruled out. In patients with a suspected diagnosis of CDI in IBD, stool studies for CDI are sent and empiric treatment is started. ELISA is the most commonly used method of diagnosis of CDI. Treatment may be started awaiting results of assay (vancomycin 125 mg orally every 6 h with continuation of their previous mmunosuppressive therapy). No new immunomodulators or escalation of immunosuppressive medications should be done in patients with suspected CDI in IBD unless CDI is ruled out with serial stool studies (at least 3-4). The duration of antibiotic use is 14 days. Routine endoscopy is not necessary as the yield of pseudomembranes is very low unless an alternative diagnosis such as cytomegalovirus infection is being entertained. Patients should be serially followed to study the impact of CDI on the short term and long term outcome of IBD.

Cytomegalovirus (CMV) is a member of the Herpesviradae family. It is transmitted through close personal contact with body fluids, including saliva, urine, blood, breast milk, semen, and transplanted organ tissue[97]. CMV infection is ubiquitous in developed nations, with laboratory evidence of prior infection in 40%–70% of the general adult population[98,99]. Immunocompromised patients may have severe end-organ involvement as a presentation of primary acute CMV infection[97,100]. Conversely, immunocompetent individuals with primary CMV infection are usually asymptomatic. In this setting, primary CMV infection is generally self-limited, and resolves to a state of lifelong latency. In the latent phase, the viral genome exists in an episomal circular form and does not replicate[101]. Latent infection does not increase

morbidity or mortality. Immunosuppression can reactivate CMV replication and cause its migration to inflammed tissue to further propagate infection[102,103]. Ample evidence in literature suggests that there is increased colonic CMV reactivation in ulcerative colitis patients[98,100, 104-107]. There are conflicting reports on whether or not CMV reactivation is associated to immunosuppression (steroids, cyclosporine or biologicals). Steroid refractory disease has also been incriminated as one of the risk factors for CMV reactivation[104,105].

Accurate and efficient diagnosis of active CMV reinfection remains a challenge. CMV-specific testing has been unreliable in the diagnosis of active CMV colitis. CMV serology, antigen testing, and DNA testing, though useful in identifying prior exposure history, can be difficult to interpret in the setting of active infection and results correlate poorly with active disease[113,114].

Table 2: Summary of diagnostic techniques and its characteristics for CMV infection[115-123]

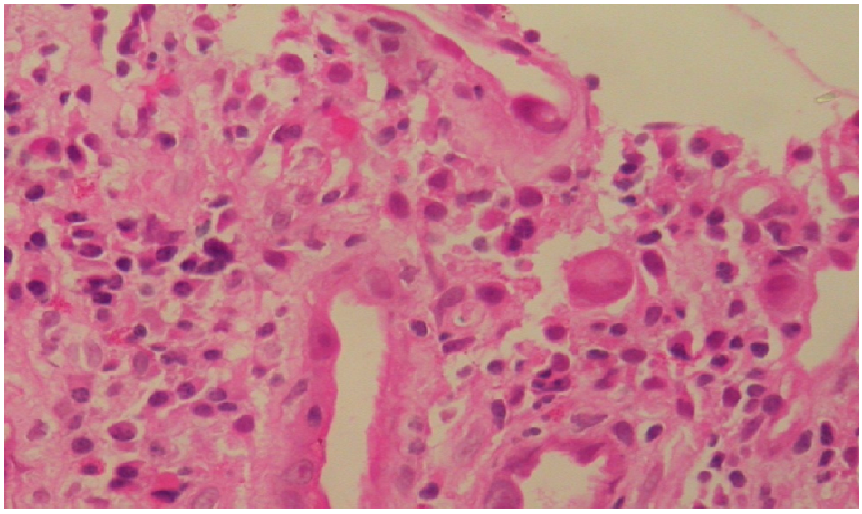
Diagnostic test	**Pros**	**Cons**	**Sensitiv -ity(%)**	**Specifici -ty(%)**
Serum serology	Can able to detect prior infection	Unable to detect active colitis	98-100	96-99
Antigen testing	Reasonable sensitivity and specificity for active disease	Semi-quantitative, results subjective	60-100	83-100
Culture	High specificity	Long incubation; poor sensitivity	45-78	89-100
DNA PCR (serum)	High negative predictive value; may correlate with active disease	Different assays and quantitation methods; low specificity	65-100	40-92
Histology(H & E)	Inexpensive stain	Poor sensitivity	10-87	92-100
Immunohistology	Improves sensitivity over H & E stain	Expensive stain	78-93	92-100

Histological demonstration of classical cytopathic changes is the current gold standard for diagnosis of CMV reactivation.

Many studies have shown the CMV reactivation as a risk factor for increased morbidity and mortality.. In a prospective observational study involving 114 patients, it was found that among steroid refractory UC, 3 of 6 (50%) patients with evidence of CMV reactivation proceeded to colectomy, compared to 2 of 13 (15%) steroid-refractory patients without CMV[104]. In another Indian study of 63 patients, 61 of which were UC patients, 4 of 10 (40%) patients with CMV required surgical intervention compared to 4 of 53 (7%) UC patients without CMV. Furthermore, 3 of 10 (30%) UC patients with CMV had fatal outcome, compared to 0 of 53 (0%) UC patients without CMV[124]. Early detection of CMV reactivation in UC patients enables prompt therapeutic intervention with IV ganciclovir or forscarnet, which has been shown to improve clinical outcomes. However, there are investigators who believe that CMV could just be an innocent bystander and has no role in acute exacerbation of UC.

Despite the burning debate about the non-pathogenicity of CMV reactivation, current literature indicates that UC patients are at higher risk for CMV reactivation, and that the failure to appropriately diagnose and treat these patients may lead to significant morbidity and mortality. This higher risk is likely multifactorial involving both iatrogenic immunosuppression and underlying factors such as severe inflammation, malnutrition and inherent immunogenicity. However, the mechanisms behind these factors have not been fully understood. Patients presenting with severe and/or refractory UC, especially patients with significant steroid use history should be evaluated for possible CMV reactivation as a possible contributor to their symptoms.

Colonic mucosa of a patient with ulcerative colitis showing crypt branching, disarray and chronic inflammation



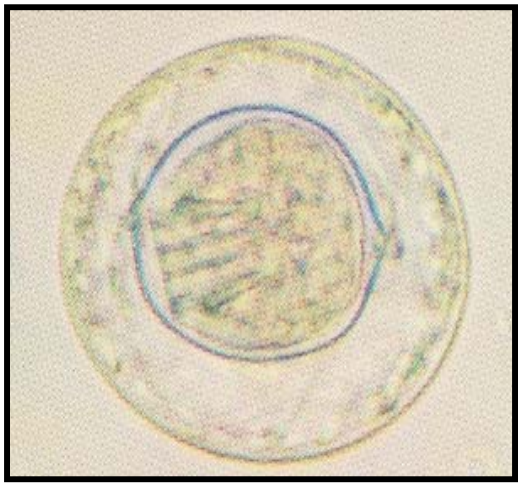
Colonic mucosa of a patient with CMV infection showing enlarged stromal cells containing intranuclear smudged inclusions

Figure 2: Biopsy changes in ulcerative colitis and co-infection with cytomegalovirus

GIARDIA CYST

STRONGYLOIDES LARVA

HOOKWORM EGG



HYMENOLEPIS NANA EGG

Figure 3: Common parasite co-infections in Ulcerative Colitis

METHODS

The study was a prospective cross-sectional observational study. The study protocol and consent form (Appendix) were approved by the Institutional Review Board (IRB)

Setting:

Department of Gastrointestinal Sciences, Christian Medical College, Vellore.

Period of recruitment:

January 2009 to January 2011

Participants:

Inclusion Criteria

- All cases of ulcerative colitis who were seen as indoor or as indoor patients were included in the study. The diagnosis of ulcerative colitis was established on the basis of Asian consensus criteria (Ooi et al[125]). The diagnosis of confirmed cases of ulcerative colitis was based on detailed clinical history, physical examination, typical colonoscopic findings and histology. Typical history included bleeding per rectum with mucus or bloody diarrhea presently or in the past. Colonoscopy appearance typical of ulcerative colitis was considered to be symmetrical and continuous inflammation starting from the rectum, extending proximally without interruption with features of loss of normal vascular pattern, erythema and edema of mucosa with or without granularity, friability with presence of yellow brown mucopurulent exudates associated with mucosal ulceration(which can be punctate, annular, linear or serpigenous), with or without the presence of pseudopolyps. Histology showing cryptitis or crypt abscesses and absence of granulomas was considered typical. Histologically the disease was graded as quiescent,

mild, moderate and severe depending on the activity. Quiescent colitis was characterized by presence of architectural alterations with or without the presence fibrosis with no features of neutrophils infiltration, no edema no ulcers. The disease was graded histologically as mild, moderate or severe according to the degree of neutrophil infiltration of the crypts and depth of mucosal infiltration, amount of mucin depletion and the amount of edema and congestion with or without presence of the ulcers.

Exclusion criteria

- Failure to provide consent
- Alternative diagnosis established after initial evaluation

Variables:

Ulcerative colitis – disease activity graded as mild, moderate or severe as per Truelove Witts criteria

Measurements:

- Usual indicated clinical and laboratory evaluation including CBC, ESR, CRP, Ca, P, LFT
- Ileo-Colonoscopy and segmental biopsy
- 3 fresh samples of stool were analyzed for parasites (including special stains)
- Stool ELISA for *Clostridium difficile* toxin

STUDY PROTOCOL

Consecutive patients were included in the study. Patients were formally interviewed and examined by the principal investigator. The patient was explained about the disease and was advised to undergo colonoscopy with segmental biopsies which is part of the current standard of

care. The clinical profile was scored according to disease activity scale – Truelove-Witts. Stool samples to look for parasites were collected on at least three consecutive days. One stool sample was analysed for ELISA for *Clostridium difficile* toxin. Segmental colorectal biopsies were looked at for evidence of parasites and for changes suggestive of CMV infection (classic owl-eye inclusion bodies).

The methods followed for stool examination for parasites are detailed below:

STOOL ROUTINE PARASITES:

Wet preparation – A drop of normal saline was placed on the slide. A uniform stool suspension was made and a coverslip was placed gently without creating any air bubbles. The thickness of the preparation was such that printed material could be read through it. The stool was examined under the microscope so as to cover all areas of the slide.

Iodine preparation – A drop of iodine was placed on the slide. A uniform stool suspension was made and a coverslip gently placed over it. The slide was systematically scanned using the 10x and 40x objectives of the microscope.

In a wet preparation, motile trophozoites, larvae, cysts and ova can be identified. In an iodine preparation, internal nuclear characters of protozoan cysts can be seen.

Formol ether concentration technique was used for analysing the stool sediment after concentration. The following was the method used. The stool was mixed well in the container using a clean stick. Approximately 1 gram of faeces was selected to include external, internal and mucus portions of the sample. The sample was placed in a centrifuge tube, containing 7 ml of 10 % formalin. The faeces was emulsified in formalin. Following this, the emulsified solution was transferred to another centrifuge tube and 3

ml of ether was added. This was mixed well on a vortex mixer for 15 seconds or by hand for 1 minute. This solution was centrifuged at 3000 rpm for 1 minute. The fat plug of debris from top of the tube was cleared by ringing the sides of the tube with a stick. The top layer of supernatant was removed by quickly inverting the tube. A few drops of 10% formalin was added to the sediment and mixed well. A slide for examination was made from the sediment, using saline and iodine preparations. 10x and 40x objectives were used to examine the whole smear for ova and cysts.The following special stains were done on the stool sample on the concentration sediment

1. Modified acid fast stain
2. Modified trichrome stain
3. Rapid Field's stain

Modified acid fast stain: The stool smear was allowed to air dry, following which it was fixed in methanol for 3 minutes. The smear was stained with carbol fuchsin for 15 - 20 minutes. Following this, it was rinsed thoroughly in tap water. The stain was decolorized with 1% acid alcohol for 15 – 20 seconds. The smear was again rinsed with tap water and counterstained with methylene blue for 30 -60 seconds. After rinsing thoroughly in tap water, the smear was examined using the 100x oil objective. The modified acid fast stain is useful to identify opportunistic parasites, such as *Cryptosporidium, Cyclospora* and *Isospora*. Oocysts of *Cryptosporidium* stain an intense red colour and most will contain several prominent black granules against the blue background.

Modified trichrome stain: The smear was fixed with methanol for 5 minutes. The smear was stained with chromotrope stain for 60 minutes at room temperature or for 10 minutes

at 50°C. The slide was rinsed thoroughly in tap water. Then, the smear was destained in acid alcohol for only 1-2 seconds. The slide was then rinsed in 95% ethanol by dipping it in for 1-2 seconds. Then it was dehydrated with 95% ethanol for 5 minutes and then in 100% ethanol for another 5 minutes. The slide was dipped in xylene for a few seconds. The slide was dried and then examined under the 100x oil objective to identify Microsporidia (belonging mainly to the genera *Enterocytozoon* or *Encephalitozoon*). Microsporidial spores stain a pinkish – red color; 1.5 – 2 μm with a clear vacuole and membrane fold.

Rapid Field's stain:

A thin film of faeces was made on the microscopic slide and was allowed to air dry. The slide was fixed with methanol for 1 minute. The slide was flooded with 1 ml of Field's stain B (diluted 1: 4 with distilled water). An equal volume of undiluted Field's stain A was added and mixed well. The slide was allowed to stand for a minute. Then it was rinsed well in tap water and drain dried. The smear was examined under the 100x oil objective of the microscope. The Rapid Field's stain is useful for detection of trophozoites and cysts of *Giardia, Blastocystis hominis* and *Dientamoeba fragilis* Flagella, cilia and nuclei stain red. Cytoplasm stains bluish-grey.

Stool for *Clostridium difficile* ELISA:

The ELISA kit used was the ProSpecT™ Microplate assay (Thermo Fisher Scientific Remel Products, Lenexa, Kansas, USA). A microplate ELISA assay, it detected both *C. difficile* Toxin A and B. The results were read by a microplate reader. An optical density ≥ 0.080 was considered as positive. Compared to tissue culture cytotoxicity assay,

specificity was 96.2% and sensitivity was 90.3%. The analytical sensitivity for toxin A and toxin B was respectively $\geq$ 0.20 ng/ml and $\geq$ 0.61 ng/ml.

COLONOSCOPY AND SEGMENTAL BIOPSY:

Olympus CF V70L or 150L was used for colonoscopy. After consent and explanation of procedure, the colonoscope was inserted upto the ileum. A withdrawal time of 7 minutes was minimum for a proper evaluation of the entire colon. Segmental biopsies were obtained from ileum, caecum, ascending, transverse colon, descending, sigmoid colon and rectum were collected in separate bottles.

SAMPLE SIZE CALCULATION:

We assumed that 55 % of our patients would have moderate to severe UC. We further assumed that the risk of having one of these organisms(bacteria, virus or parasites) would be 0.25 in the severe group and 0.05 in the mild group. We analysed and found that we needed 50 patients in the moderate-severe UC group and 37 patients in the mild group to be able to reject the null hypothesis that infection with pathogens is not associated with disease activity with probability(study power) 0.8 and a type I error probability of 0.05.

STATISTICAL METHODS:

SPSS version 15.0 was used to analyse data. Frequencies of variables were calculated. Mean and range were calculated for continuous data. Chi-square test (Fisher exact test) was used to find out correlation between UC activity and presence of parasites, CD toxin or CMV positivity.

RESULTS

Demographic characteristics:

A total of 87 patients were included in the study. 51(59%) were male and 36(41%) were female. 70 % of patients hailed from the eastern part of India. Mean age of our patients was 40.2 ± 12 years (Range: 16-69 years). Mean body mass index was 22.2 ± 4.4 (Range: 12.5-39.1). Majority of our patients were Hindu (78.2%)

Figure 4: Sex distribution of cases (*n*=87)

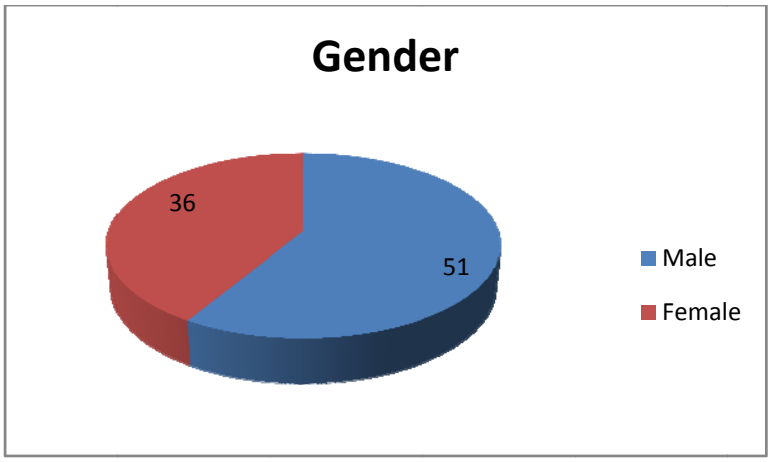


Figure 5: Geographical distribution (*n*=87)

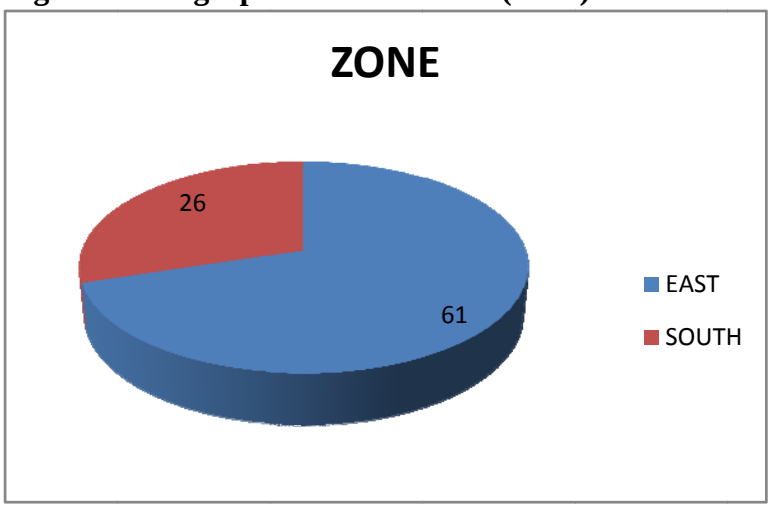
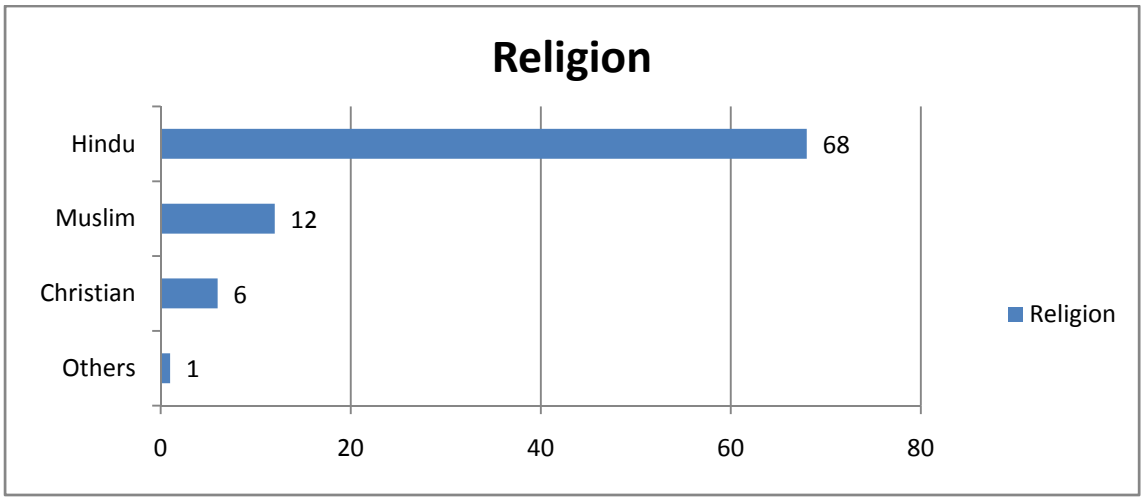


Figure 6: Religion distribution (*n*=87):



Age of onset of symptoms:

Mean age of onset of symptoms of ulcerative colitis was 35.2 ± 12.6 years (Range: 13-67)

Table 3: Age distribution at onset of symptoms

Age range	Number of patients	Percentage(%)
11-20	12	13.8
21-30	25	28.7
31-40	23	26.4
41-50	14	16.1
51-60	11	12.6
61-70	2	2.4
Total	87	100%

Symptoms:

The following were the symptoms of ulcerative colitis noted in our patients.

Table 4: Symptoms in ulcerative colitis patients

Symptoms	Number of patients	Percentage
Diarrhea	82	94.3
Bloody stools	83	95.4
Abdominal pain	41	47.1
Urgency	74	85.1
Incontinence	48	55.2
Tiredness	71	81.6
Fever	16	18.4
Weight loss >5 kg	64	73.6
Joint involvement	36	41.4
Eye involvement	1	2.3
Skin involvement	0	0

Most common symptoms in ulcerative colitis noted in our study were bloody stools (95.4%) and diarrhea (94.3%). Urgency and incontinence were seen in 85.1% and 55.2% of patients respectively. Arthritis was noted in 41.4% of our patients. Only one patient(2.3%) had eye involvement in the form of iritis.

Past history:

Table 5: Past and family history: (*n*=87)

	Number	Percentage
Diabetes mellitus	10	11.5
Hypertension	11	12.6
Tuberculosis	5	5.7
Appendicectomy	5	5.7
Family history	5	5.7

10(11.5%) of our patients had diabetes mellitus, 11(12.6%) of our patients had hypertension. History of appendicectomy was present in 5(5.7%).

5.7 % had a first or second degree relative having ulcerative colitis.

Personal history:

Table 6: Personal History:

	Number of patients	Percentage
Smoking	11	12.6
Tobacco chewing	19	21.8
Alcohol	2	2.2
Non vegetarian diet	80	92

11 (12.6%) of our patients were smokers. 19(21.8%) had a habit of tobacco chewing. Majority of our patients were non-vegetarian (92%).

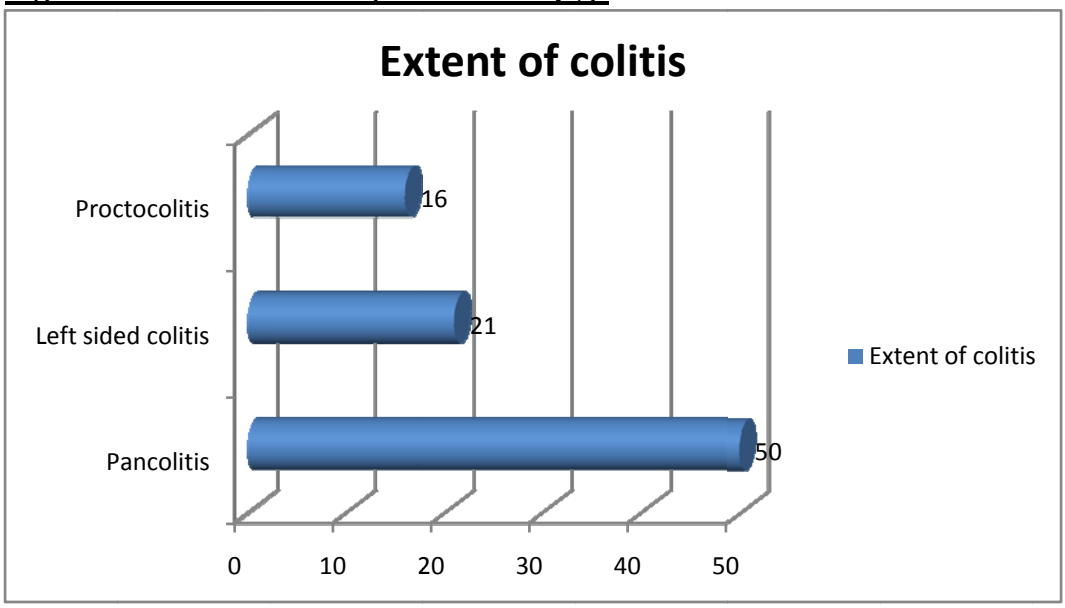
Treatment history:

Table 7: Treatment history

	Number of patients	**Percentage**
Present 5-ASA use	64	73.6
Past 5-ASA use	62	71.3
Oral steroid use	36	41.4
Rectal steroid use	19	21.8
IV steroid use	2	2.3
Azathioprine use	15	17.2

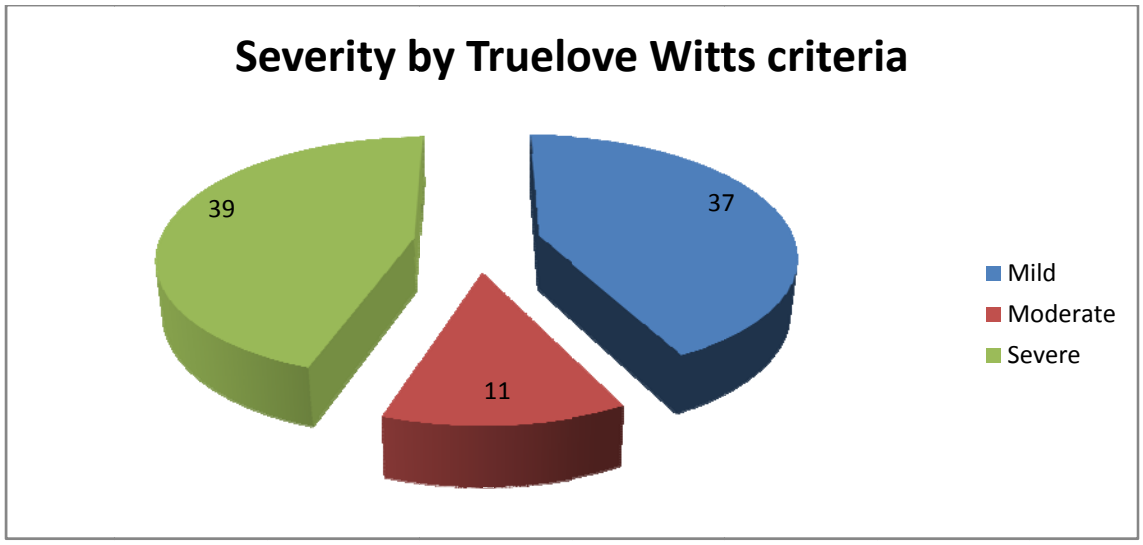
Most of our patients were on treatment when they were enrolled for the study. The most commonly used agent was 5-ASA (aminosalicylic acid) (73.6%). Oral steroids were being used by 41.4% of our patients. 17.2% of our patients were on azathioprine.

Figure 7: Extent of colitis: (At colonoscopy):



57.4% of patients had pancolitis, 24.1% had left sided colitis and 18.3% had disease limited to the rectum

Figure 8: Truelove Witts score:



39(44.8%) patients had severe disease, 11(12.6%) had moderate and 37(42.6%) had mild disease by Truelove Witts clinical scoring.

Laboratory parameters:

Baseline laboratory parameters were as under

Table 8: Lab parameters:

	Mean ± SD (Range)
Haemoglobin (g%)	11.7 ± 2.4 (5.8-19)
ESR	41.2 ± 29.2 (2-125)
Albumin(g%)	4 ± 0.9 (0.9-5.2)

Biopsy activity:

Majority of patients (58.6%) had moderate inflammation UC on biopsy, 11.6% had severe and 29.8% had mild activity.

Table 9: Biopsy activity:

	Number of patients	**Percentage**
Mild	26	29.8
Moderate	51	58.6
Severe	10	11.6

Pathogens detected:

Pathogens (either of parasites, cytomegalovirus or *Clostridium difficile* toxin) were found in 17.2% of patients. 10(11.5%) of patients had a parasitic infestation.

Table 10: Pathogens detected :

	Number	**Percentage**
Parasites	10	11.5
Cytomegalovirus	2	2.3
Clostridium difficile toxin	3	3.4

Cytopathic changes on colonic biopsy were found in 2 out of 87(2.3%) patients. Both had severe UC and had to be hospitalized for treatment. In both patients, the colonic mucosal biopsy PCR as well as blood mononuclear cell PCR were positive for CMV. One of them responded only partially to ganciclovir and subsequently required subtotal colectomy while the other improved with ganciclovir and medical therapy for ulcerative colitis.

Clostridium difficile toxin in stool was detected in 3 out of 87(3.4%) patients. All the three had severe UC as per Truelove-Witts criteria. Prior antibiotic use was present in two out of three patients

Parasites:

Table 11: Parasites: (n=10)

Parasite	**Number**
Giardia cysts	5
Strongyloides larva	3
Hookworm ova	2
Hymenolepis nana ova	1

Note: One patient had infection with both strongyloides and hookworm.

The most common parasite noted was giardia (5 patients), followed by strongyloides (3 patients).

Presence of pathogens by clinical severity (Truelove Witts criteria):

Table 12: Correlation of severity of UC with pathogens

Truelove Witts score	**Pathogen present**	**Pathogen absent**	
Moderate/severe UC	13	37	50
Mild UC	2	35	37
	15	72	87

(***p* value=0.02** Two-tailed Fisher exact test)

The presence of pathogens was very significantly associated with moderate/severe UC. 13 out of 15 cases (86%) with pathogens had moderate to severe UC while 37 out of 72 cases (51%) without pathogens had moderate to severe UC.

DISCUSSION

Ulcerative colitis (UC) is a chronic, remitting-relapsing type of disease. This implies that the patient must take medication for very long periods. The treatment of inflammatory bowel disease (IBD) is essentially immunosuppressive. In a tropical country like India where the incidence of infective colitis is very high, it is essential to demonstrate the presence of pathogens in stool in acute exacerbations and relapses. Treating the infection may save the patient from risks of hiking up the immunosuppression.

The age of onset of symptoms of ulcerative colitis was mostly in the second and third decade of life. This was in keeping with previous studies (Yang SK et al and Probert CS et al) done in this regard[126, 127]. Both these studies described an almost equal sex distribution. Our study had a predominance of males, probably due to referral bias.

Table 13: Clinical characteristics of our patients in comparison to previous studies

Characteristic	**Jiang XL et al, 2002[128]**	**Park SH et al, 2007[129]**	**Present study**
Male: Female ratio	1.09	0.94	1.42
Mean age at onset(yrs)	40.7	40 (Median)	35.2 $\pm$ 12.6
Proctitis	70.2%	44.1%	18.4%
Left sided colitis	22.5%	22.7%	24.1 %
Pancolitis	7.3%	33.2%	57.5 %
Disease clinical severity			
Mild	-	49%	42.6%
Moderate	-	41.1%	12.6%
Severe	-	8.6%	44.8%

The higher number of severe cases in our centre could be attributed to referral bias.

Parasites, cytomegalovirus and Clostridium difficile in ulcerative colitis:

The reported incidence of such infections among IBD patients varies between 9 and 13%. A recent report from AIIMS found that 12% of patients with active UC had parasites, 8% had CMV and 10% had HSV infection[73]. The clinical presentation of an acute episode in a majority of UC patients is indistinguishable from enteric infection. Patients with ulcerative colitis often have intermittent flares alternating with periods of quiescent disease. Intestinal infections may be responsible for flares in some of these patients. Infection with intestinal parasites such as *Entamoeba histolytica* and *Strongyloides stercoralis* is likely to cause flares in ulcerative colitis. Less commonly clinically recognized are infection with cytomegalovirus and *Clostridium difficile*. In a study from Chandigarh, *C. difficile* toxin was detected in the stool of 13% of patients with UC[74]. In an earlier study from northern India, 32% of active UC patients had parasitic or bacterial pathogens, compared to 4% of inactive UC patients[75].

The lower frequency of *Clostridium difficile* in our study may be related to our use of single stool test ELISA for toxin. Four samples on consecutive days tested for *Clostridium difficile* toxin could yield a sensitivity of 92%[89]. However in the study from Chandigarh by Vaishnavi et al[74], only one stool specimen was tested for *C. difficile* toxin and still the frequency of detection was 13%. Regional variations could partly explain the lower frequency of CDT positivity, we observed in our study.

We diagnosed cytomegalovirus only by cytopathic changes on histopathology analysis. However, a previous study by Banerjee et al[73] had used both biopsy and CMV PCR from tissue for the same, which explains the lower yield of cytomegalovirus in our study. However, although 8% patients were positive for CMV PCR in tissue in Banerjee's study in comparison to our

2.3%, none of the patients were treated as they failed to demonstrate cytopathic changes on biopsy. In our institution we have a policy of treating ulcerative colitis patients for cytomegalovirus only when cytopathic changes, positive biopsy PCR and positive blood PBMC PCR (peripheral blood mononuclear cell polymerase chain reaction) are all positive. We could recognize two patients on biopsy, both of whom were positive for all three tests, and of whom one improved with ganciclovir and the other showed only temporary improvement eventually requiring surgery.

The following is a graphical representation of our data in comparison with previously done studies in this regard.

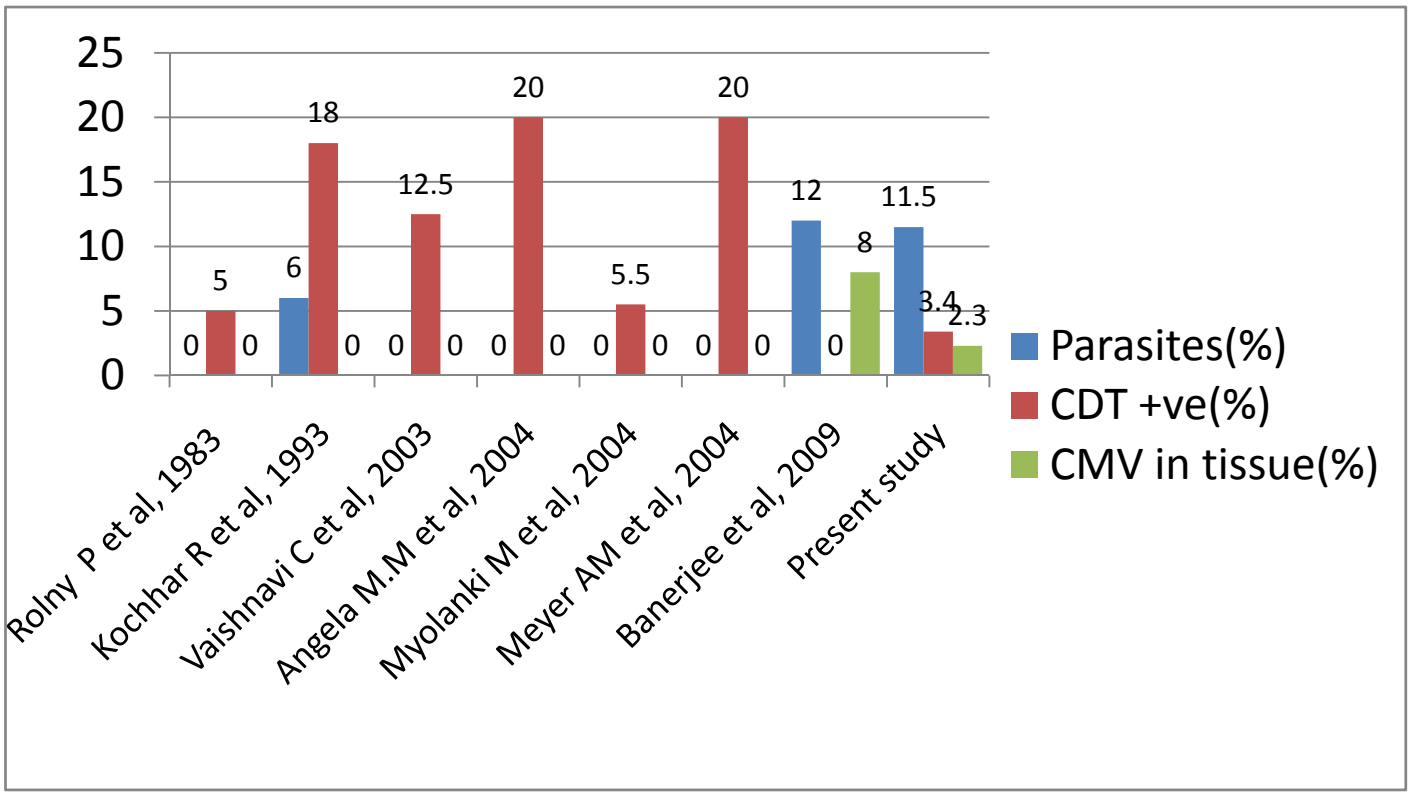


Figure 9: Comparison of yield of pathogens with previously done studies

We found in our study that the presence of pathogens was very significantly associated with moderate/severe UC. 86% with pathogens had moderate to severe UC while 51% without pathogens had moderate to severe UC.

There is no specific persistent infection described in literature which causes IBD. But enteric pathogens could set the ball rolling for initiation or reactivation of quiescent disease[130]. Though most enteric infections are self-limited, these infections could initiate a cascade of inflammatory events leading to a chronically inflammed state or a relapse of inflammatory bowel disease in a genetically susceptible host (hit and run hypothesis). Thus, enteropathogenic microorganisms play a substantial role in the clinical initiation and relapses of IBD.

Kochhar et al[75] identified 1 patient with trophozoites of *Entamoeba histolytica* in 25 patients with active UC. Another study by Mylonaki et al[131] identified 5 out of 213 patients (3 *Entamoeba histolytica,* 1 each with strongyloides larvae and *Blastocystis hominis*). Banerjee's study[73] from AIIMS reported a frequency of parasites to be 12% in their patients with active colitis. We demonstrated 10 patients having parasites out of 87 patients (13.8%) with UC. 8 out of 10 patients had moderate–severe disease by Truelove Witts criteria.

The relationship of parasitic infection to inflammatory bowel disease has evoked much interest since the recognition that certain helminth infections can be used to treat some patients with Crohn's disease or ulcerative colitis[132]. Helminth parasites modulate the immune reaction and change it from a Th1 over-reaction to a Th2 type of reaction. This is effective therapy in a minority of patients with Crohn's disease which is known to be a Th1 over-expressing disease. The pathogenesis of ulcerative colitis is much less clear and Th17 pathways may be involved. It is not yet known how much or in which direction the immune reaction (that is responsible for

inflammation) is altered by protozoal parasites (such as Giardia) or helminthes (such as Strongyloides). Nevertheless, finding such associations as we found in this study are the first step to elucidating the immunopathology of this interaction.

CONCLUSIONS

- The presence of pathogens (parasites, cytomegalovirus and/or *Clostridium difficile*) was very significantly associated with moderate/severe UC (*p* value = 0.02).
- 86% of patients testing positive for the above pathogens had moderate to severe UC while 51% of patients without pathogens had moderate to severe UC.
- Ten patients (11.5%) had a parasitic infestation. Eight out of these ten patients had moderate to severe disease.
- Three patients (3.4%) had *Clostridium difficile* toxin positivity in stools. All had severe disease. They improved with medical treatment.
- Two patients (2.3%) had cytopathic changes suggestive of cytomegalovirus infection on biopsy. Both had severe disease. One of them responded only partially to ganciclovir and subsequently required subtotal colectomy while the other improved with ganciclovir and medical therapy for ulcerative colitis

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PATIENT'S INFORMATION SHEET AND CONSENT

Study title: Correlation between severity of ulcerative colitis & co-infection with parasites, cytomegalovirus and/or *Clostridium difficile*

Principal Investigator: Dr. Venkatakrishnan H Iyer

INFORMATION SHEET (to be read by, or explained to the patient)

Acute episodes of ulcerative colitis are known to be precipitated by intestinal parasites, CMV and clostridium difficile. Dr. Venkatakrishnan is conducting a study to know how frequently these parasites and bad bacteria and viruses are present in ulcerative colitis, and whether this is contributing to the worsening of the disease.

You are being asked to provide some information about your illness. The tests that are done are mostly those are absolutely required for your medical care, and include giving three stool samples for examination. In addition, you will have colonoscopy and biopsy which is also part of the normal care for your disease. If your biopsy shows the possible presence of virus, then you will be asked to undergo further tests, which again are part of the clinical care of your disease.

The results of the tests done in connection with the study are very likely to directly benefit you. The information that we collect from the study will benefit other patients with the disease.

The results of the tests will be kept confidential and there will be no direct link of the test report with your hospital records.

You are free to not participate in this study if you do not so wish, and this will not affect your care in any way.

CONSENT

I hereby provide my consent for the above research study and to give the necessary information and samples.I also provide my consent for the stool samples to be preserved for additional research without my further approval, under the condition that the results will be kept anonymous and not linked to me.

Name: Hosp. No: Study No:

Date: Signature:

Witnessed by: (Name :)

PROFORMA FOR PARASITES, C. DIFFICILE AND CMV IN ULCERATIVE COLITIS

Patients Name: CMCH No:

Contact Address: Occupation:

Income/month:

Telephone:

E Mail:

1. Age in years:
2. Year of birth:
3. Gender:
4. Mother Tongue: 1.Tamil 2.Telugu 3.Malayalam 4.Kannada
   5.Bengali 6.Oriya 7.Hindi 8.Others

5. State Of Origin:

6. Weight (Kg):

7. Height (cm):

8. Mid arm circumference:

9. Religion: 1.Hindu 2. Muslim 3. Christian 4.Other

10. Education: 1. None 2. I- V Std 3. VI-X Std 4.Plus 2
    5. Graduate 6. Post Graduate

Patient History:

1. At what age did the symptoms appear?
2. At what age was the disease diagnosed?
3. Which year was diagnosis first made?
4. Where was the diagnosis made? 1.CMC 2.Local hospital 3.Other

Have you ever had the following symptoms in relation to your illness?

5. Abdominal Pain 1.Yes 2.No

6. Diarrhoea 1.Yes 2.No

7. Blood in Stools 1.Yes 2.No

8. Urgency 1.Yes 2.No

9. Incontinence 1.Yes 2.No

10. Tiredness 1.Yes 2.No

11. Fever 1.Yes 2.No

12. Intestinal Obstruction 1.Yes 2.No

13. Have you lost weight? 1. Nil 2. <5Kg 3. 5-10Kg 4.>10Kg

14. What is the frequency of your stools now?

15. Have you had anal canal lesions: 1. Fissure 2. Ulcers 3.Fistulae

16. Have you had painful boils in legs/body: 1.Yes 2.No

17. Have you had painful redness of eyes: 1.yes 2.No

18. Any other problem:

<u>Past Medical History & Family and Personal History:</u>

19. Diabetes mellitus 1. Yes 2.No

20. Hypertension 1.Yes 2.No

21. Pulmonary tuberculosis 1. Yes 2.No

22. Extra Intestinal tuberculosis 1.Yes 2.No

23. Were you ever diagnosed to have intestinal TB? 1. Yes 2.No

24. Have you had appendicectomy? 1. Yes 2.No

25. If yes, was it done for acute abdominal pain? 1. Yes 2.No

26. Have you had any other operation on abdomen? 1. Yes 2.No

27. Has any other family member had IBD? 1. Yes 2.No

28. Do you currently smoke cigarettes/beedies?

1. No 2. <7 per week 3. 7/wk to 10/day 4.> 10/day

29. In the past did you smoke cigarettes/beedies?

1. No 2. <7 per week 3. 7/wk to 10/day 4.> 10/day

30. Did you chew tobacco alone or with paan? 1. Yes 2.No

31. Do you take alcohol drinks?

1. Teetotaler 2. Social drinker 3.Moderate drinker 4.Heavy drinker

32. What diet do you take?

1. Strict Vegetarian 2.Eggs also 3.Non-vegetarian

<u>Medical treatment:</u>

33. Have you ever used aminosalicylates in the past? 1. Yes 2.No

34. Have you ever used oral steroids in the past? 1. Yes 2.No

35. Have you ever used rectal steroids in the past? 1. Yes 2. No

36. Have you ever used IV steroids in the past? 1.Yes 2.No

37. Are you currently using aminosalicylates? 1.yes 2.no 3.cannot say

38. Are you currently using steroids (any form)? 1.yes 2.no 3.cannot say

39. Are you currently using azathiprine/immunosuppressant?

1.yes 2.no 3.cannot say

40. Have you received drugs for TB in the past? 1.yes 2.no 3.cannot say

41. Are you currently taking drugs for TB? 1.yes 2.no 3.cannot say

42.Laboratory findings:

Test	Date/Finding	Report/Biopsy
Hb (g/dl)		
MCV (fL)		
ESR (mm/hr)		
CRP		
S Albumin		

C. difficile toxin		
Stool Parasites x 3		
Endoscopy		
Colonoscopy		
Colonoscopic biopsy Activity To look for any evidence of CMV		
Grade (Truelove- Witts)		
Grade(Mayo)		

Mayo index	0	1	2	3
Stool frequency	Normal	1–2/day >normal	3–4/day >normal	5/day >normal
Rectal bleeding	None	Streaks	Obvious	Mostly blood
Mucosa	Normal	Mild friability	Moderate friability	Spontaneous bleeding
Physician's global assessment	Normal	Mild	Moderate	Severe

from Truelove and Witts'[13]

	Mild	Moderate 'in between mild and severe'	Severe
Bloody stools/day	<4	4 or more *if*	≥6 *and*
Pulse	<90 bpm	≤90 bpm	>90 bpm *or*
Temperature	<37.5 °C	≤37.8 °C	>37.8 °C *or*
Haemoglobin	>11.5 g/dL	≥10.5 g/dL	<10.5 g/dL *or*
ESR	<20 mm/h	≤30 mm/h	>30 mm/h *or*
or CRP	Normal	≤30 mg/L	>30 mg/L